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Studies on Enzyme Action. XV.*—*Urease: a Selective Enzyme.*

By HENRY E. ARMSTRONG, F.R.S., and EDWARD HORTON.

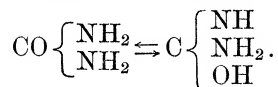
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[*International Catalogue of Scientific Literature.*

D 1310 } (Urea). Hydrolysis by urease (Soja bean).
Q 8311 }
D 1814 } Urease (Soja bean). Action on urea.]
Q 1240 }

The production of urea from the metamerie ammonic cyanate by Wöhler, in 1828, was naturally regarded as an achievement of great importance in view of the part the substance plays in the human economy—very nearly the whole of the nitrogen secreted being voided as urea; the synthesis of such a compound was a severe blow to the vitalistic conceptions which had been dominant in organic chemistry and justly excited attention, as it broke down the barriers up to that time held to be existent between the organic and inorganic world.

The attributes of urea are in many ways remarkable and scarcely in correspondence with the simple formula ordinarily assigned to it. It is generally spoken of in the text-books as *carbamide* but its properties are more nearly those of the isomeric *hydroxy-amino-imino*-derivative: an explanation may be found in the assumption that the two isodynamic substances are present in an aqueous solution in oscillatory equilibrium:—



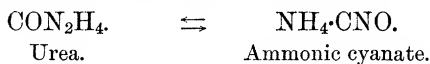
But it is probable that these two compounds, in turn, are in equilibrium with the hydrate common to both, $\text{C}(\text{NH}_2)_2(\text{OH})_2$: as chemical changes are all reciprocal effects, the behaviour of urea is therefore subject to considerable variation and depends on the conditions under which it is placed; on this

* XIV, 'Annals of Botany,' 1911, vol. 25, pp. 507—519. I—XIII, these 'Proceedings,' Series B.

account, a name which is significant of the origin of the substance rather than of its functions is specially appropriate.

Equilibration of Urea and Ammonic Cyanate in Solution.—Though the conception that salts in solution must be pictured as in a state of constant flux was advanced by A. Williamson, in 1850, in his celebrated memoir on the Theory of Etherification, little notice was taken of the suggestion and the phenomena of reversible change were not taken seriously into account until Deville's work on dissociation at high temperatures attracted attention; since that time it has been realised gradually that the properties of solutions are such as to necessitate the general application of Williamson's contention.

Urea is a remarkably stable substance in solution and is but slowly hydrolysed even by heating it with acids and alkalis.* At ordinary temperatures no perceptible change takes place in the solution: that is to say, it remains neutral and does not contain an appreciable amount of ammonia; on this account, probably, the alteration it undergoes was overlooked until J. Walker and F. J. Hambly† drew attention to the fact that urea and ammoniac cyanate are related reversibly, thus:—



The conversion into the cyanate is easily demonstrated. No precipitate is noticeable on adding nitrate of silver to a cold solution of urea; if, however, the solution be heated to 80°—100°, then quickly cooled and mixed with the silver salt, cyanate of silver is at once thrown down; if equivalent quantities of the two substances are heated together, a very large proportion of urea is converted into cyanate, indeed the conversion may be rendered almost complete by concentrating the solution.

The silver precipitate contains only traces of carbonate when urea is used initially, but when ammoniac cyanate is transformed into urea, a considerable proportion—as much as about 4 per cent.—is converted into carbonate.

When equilibrium is established, a solution of urea which has been heated at 80°—100° appears to contain at most about 5 per cent. of the metameric cyanate and at lower temperatures the proportion is much less.

According to Walker and Hambly, the rate at which ammoniac cyanate undergoes conversion into urea is such that it cannot be supposed that only a single changing substance is present in the solution, the inversion proceeding at a "bimolecular" rate: in other words, the single molecule of cyanate is not changed directly into the single molecule of urea. According to Fawsitt, the hydrolysis of urea follows the unimolecular law.

* Comp. Fawsitt, 'Zeit. Phys. Chem.,' 1902, vol. 41, p. 601.

† 'Chem. Soc. Trans.,' 1895, p. 746.

The observed rate of change may be accounted for in two ways: either on the assumption that when the cyanate is dissolved in water it is in part resolved into cyanic acid and ammonia; or on the assumption that the cyanate is dissociated electrolytically into the two ions, the ammonic ion and the cyanic ion—the process measured in either case being the rate at which the reverse interaction takes place by which the cyanate is reconstituted and transformed into urea.

Walker and Hambly have proved that whereas a neutral salt such as potassic sulphate and also ammonia have but a slight retarding effect, ammonic sulphate and potassic cyanate both hasten the rate at which cyanate is changed into urea.

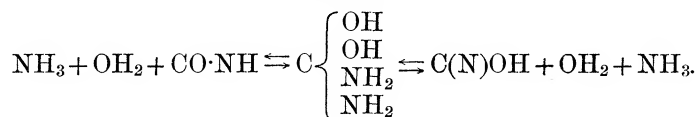
The same observers have shown that a variety of non-electrolytes—methylic and ethylic alcohol, glycol, glycerol, acetone and cane sugar—have also a marked influence in accelerating the rate of change; they express themselves unable to account for the acceleration, though they have no doubt as to the interpretation to be given to the results they obtained with electrolytes, as they remark “On no other theory (than that of electrolytic dissociation), as it appears to us, can even a qualitative explanation of our results be given.”

We venture to differ. To us it appears easy to interpret the results on ordinary simple principles without invoking the aid of a mysterious, electrolytic-ionic dissociation. It is sufficient to assume that ammonic cyanate undergoes hydrolytic dissociation (dissociation through the agency of water) into cyanic acid and ammonia and that urea is formed by the gradual interaction of these substances and water; the effects electrolytes produce in altering the rate of change may be accounted for as easily on this assumption as on that of ionic dissociation favoured by Walker and Hambly. The fact is the observations may be interpreted from either point of view: as in so many other cases, the ionic explanation is merely an ornate paraphrase of the ordinary chemical point of view.

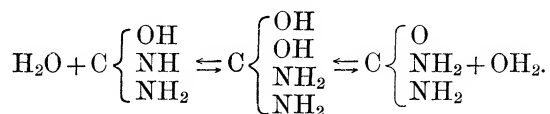
Walker and Hambly offer no explanation of the formation of urea. It appears from their observations that when ammonic cyanate is dissolved in water it is to some extent resolved into its components; the slowness with which urea is formed at moderate temperatures is attributable, it may be supposed, to the slowness with which cyanic acid, ammonia and water interact and with which the product of their interaction gives rise to urea, the slowness arising perhaps from the frequent reversals which attend the changes. In any case, the process is to be regarded as one of considerable complexity, in no way as a mere structural change in a single molecule.

The uncertainty we feel as to the structure of cyanic acid is of little

consequence in such a discussion, as it is probable that a solution of the substance contains both metameric forms of the compound and that each of these can pass over into the other as required. Such molecules would be converted by the combined action of ammonia and water into a dihydroxy-diamino-methane or diamino-methanediol:

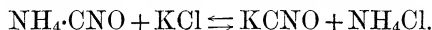


The dehydration of the diol might well occur in either of two ways and give rise to one or other of the two forms of urea:



Such dehydration changes would take place, we may suppose, the more readily the more concentrated the solution and the higher the temperature; they would be promoted by the presence of salts, as these would "concentrate" the solution; and would also be promoted by non-electrolytes, as these would raise the osmotic tension in the solution and render the water itself more active as a dehydrating agent.

Ammonic salts and cyanates, in common with all other salts, would act as "dehydrating" agents and therefore as accelerators. The effect produced by salts other than these, however, would be two-fold: whilst active as accelerators, they would also tend to confer stability on the cyanate, in virtue of the occurrence of changes such as the following:—



Ammonia, being so very weak a base, would have a relatively slight effect in promoting the formation of the diamino-derivative and increasing the amount of change; on the other hand, by increasing to some extent the activity of water, it would to some extent perhaps also counteract its own tendency to increase the proportion of diamino-derivative produced, whilst it would in some degree promote dehydration changes. It may be expected, therefore, that the aggregate effect of ammonia would be slight.

Hydrolysis of Urea by Soja Urease.—It is well known that though urea does not undergo hydrolysis under ordinary conditions, it rapidly disappears from voided urine—the reason being that organisms which cause its destruction soon develop in the liquid. The hydrolysis is affected by *urease*, an enzyme present in *Micrococcus urae* and a number of other organisms. The

literature of the subject is very fully brought together and discussed in Reynolds Green's 'Soluble Ferments and Fermentation' and similar works.

No simpler case of hydrolysis by an enzyme than that of urea is known. On this account we have long desired to include the interaction of urea and urease in our series of studies of enzymes. At various times, we have attempted to obtain the necessary enzyme for the work but the difficulties we encountered have been greater than we anticipated: little progress was made, therefore, until we were able to avail ourselves of the discovery made by Takeuchi, in 1909,* that the Soja bean (*Glycine hispida*) is particularly rich in urease.

Preparation of Enzyme.—To prepare a solution of the enzyme, the Soja beans are twice ground in a coffee mill and the meal extracted with petroleum spirit, the extraction being repeated six times; usually the petroleum is left in contact with the meal during about 45 minutes and then removed by filtration under reduced pressure. The operation is carried out in a large glass separating funnel. The washed meal is spread out during a night on glass plates to allow adherent petroleum to evaporate. Next morning it is mixed with five times its weight of water and a little toluene. After 24 hours, the liquid is separated from the meal by filtration through a large Buchner funnel; from 60 to 70 per cent. of liquid is recovered.

The solution thus prepared has a considerable degree of activity, 20 c.c. mixed with an equal volume of a solution containing about half a gramme of urea sufficing to hydrolyse the urea within half an hour at 20°.

The solution contains a large amount of albuminous matter. The endeavour has been made to purify the enzyme by precipitating with alcohol and extracting the precipitate with water; as the extract prepared in this manner is very weak in comparison with the new extract, we have preferred to use the latter.

The fresh extract is faintly acid; when kept, it increases in acidity and a coagulum is deposited; at the same time, its hydrolytic activity falls, the fall being considerable when the precipitation sets in. The changes that take place in the activity of the solution will be considered later on.

Method of Determining Activity of Enzyme.—We have found the simplest method of determining the activity of urease to be to digest the solution of urea with the enzyme and subsequently neutralise the ammonia that is formed by an excess of decinormal acid, then boil during two to three minutes to expel carbon dioxide, finally titrating with baryta solution. The errors incidental to this method do not appear to be greater than those associated with more elaborate methods.

* 'J. Coll. Agric. Imp. Univ. Tokyo,' 1909, vol. 1, pp. 1—14.

In carrying out the experiments, as a rule, the mixtures studied were contained in Jena flasks provided with rubber stoppers. In some cases, when it was thought that ammonia might be lost by volatilisation, the stopper was provided with a short length of glass tubing to which a piece of rubber tubing carrying a screw clip was attached; the mixture having been introduced, the pressure within the flask was slightly reduced and the clip then screwed up; at the close of the experiment, by attaching the rubber tube to the burette and allowing acid to run in, it was possible to prevent any escape of ammonia. In some experiments in which a large number of samples were removed in rapid succession, the mixture was contained in a flask attached to an automatic pipette and the liquid forced up into this latter by air pressure—both flask and pipette being in the incubator and the pressure operated from outside.

Selective Activity of Urease.—Takeuchi has tested the action of Soja urease on a considerable number of nitrogen compounds, namely alanine, allantoin, arginine, benzamide, glycine, guanine, hippuric acid, histidine, kreatinine, leucine, tyrosine and uric acid; none of these was affected. He states that biuret is slightly attacked but his figures scarcely justify the conclusion.

We have thought it desirable to study the behaviour of substances more closely related to urea and have therefore subjected the various substituted ureas to the action of urease.

A convenient and striking way of demonstrating the hydrolysis of urea by Soja urease is to place a small quantity of a 5 per cent. solution of urea in a flat glass dish or on a white plate, then to add a drop or two of an alcoholic solution of phenolphthalein and dust in a quantity of the bean meal free from oil or even raw meal. In a very short time, especially if the liquid be slightly warmed, the colourless solution assumes a pink and then a full rose-red colour. If the experiment be made with either methylurea or ethylurea, no such effect is observed.

In a first series of experiments with various substituted ureas in which solutions of the ureas were digested with the solution of urease and then subjected to distillation with steam, the distillates obtained were so slightly alkaline that it was evident that little if any action had taken place. A second set of observations was then instituted with 2M/5 solutions, prepared at 25°, of methylurea, *s*-dimethylurea, *as*-dimethylurea, ethylurea and *s*-diethylurea and one of biuret prepared at 35° (as the biuret crystallised out at 25°).

From each of the solutions, 20 c.c. was measured into each of two 300 c.c. round Jena flasks, one containing 20 c.c. of Soja extract, the other 20 c.c. of water. The 12 flasks containing these mixtures and another containing

20 c.c. of Soja extract diluted with 20 c.c. of water were closed with india-rubber stoppers through which passed glass tubes provided with indiarubber tubes and screw clips. The flasks were exhausted slightly and then kept in incubators at 25°. After 24 hours, the contents of each flask was heated to boiling and distilled in a current of steam during 15 minutes, excessive frothing of the solutions containing enzyme being prevented by addition of a few drops of olive oil. Each distillate was collected in 10 c.c. of a standardised solution of chlorhydric acid; the distillate was then heated to boiling to expel carbon dioxide, cooled and titrated with baryta solution, using litmus as indicator. The results are given in Table I.

Table I.—Action of Soja Urease on Substituted Ureas.

Substituted urea.	Standard acid neutralised by distillate from		Amount of hydrolysis of		
			Urea alone.	Urea + enzyme.	
	Urea alone.	Urea + enzyme.		Uncorr.	Corrected for enzyme.
	c.c.	c.c.	per cent.	per cent.	per cent.
Methylurea	0·0	0·45	—	0·3	0·15
s-Dimethylurea ...	0·5	0·8	0·3	0·5	0·4
as-Dimethylurea ...	0·2	0·2	0·1	0·1	0·0
Ethylurea	0·6	1·65	0·4	1·0	0·9
s-Diethylurea	0·25	0·25	0·15	0·15	0·03
Biuret	0·35	0·3	0·15	0·12	0·05

The evidence thus obtained appears to us to be conclusive proof that urease is capable of acting only on urea itself—in other words, it exercises a purely selective effect and must, like other enzymes, in some way correspond very closely in structure with the hydrolyte urea with which alone it is in correlation.

The value of such correlation will be realised more fully perhaps when it is stated that either acid or alkali of normal strength may be kept in contact with urea during several days at 25° without any perceptible degree of hydrolysis being effected, though in the presence of the enzyme the urea is resolved rapidly into ammonia and carbonic acid.

Time Rate of Change of Urea by Soja Urease—Effect of Concentration.—In our earliest experiments we were struck by the fact that the graphs representing the course of the change were of extreme flatness in comparison with those representative of the action of other enzymes. On attempting to effect the hydrolysis at a constant rate by means of a very small proportion of enzyme, using M/5 solutions of urea, we found that though, in the later

periods, the amount of change taking place was approximately proportional to the time, the extent to which hydrolysis took place during the first hour

Table II.

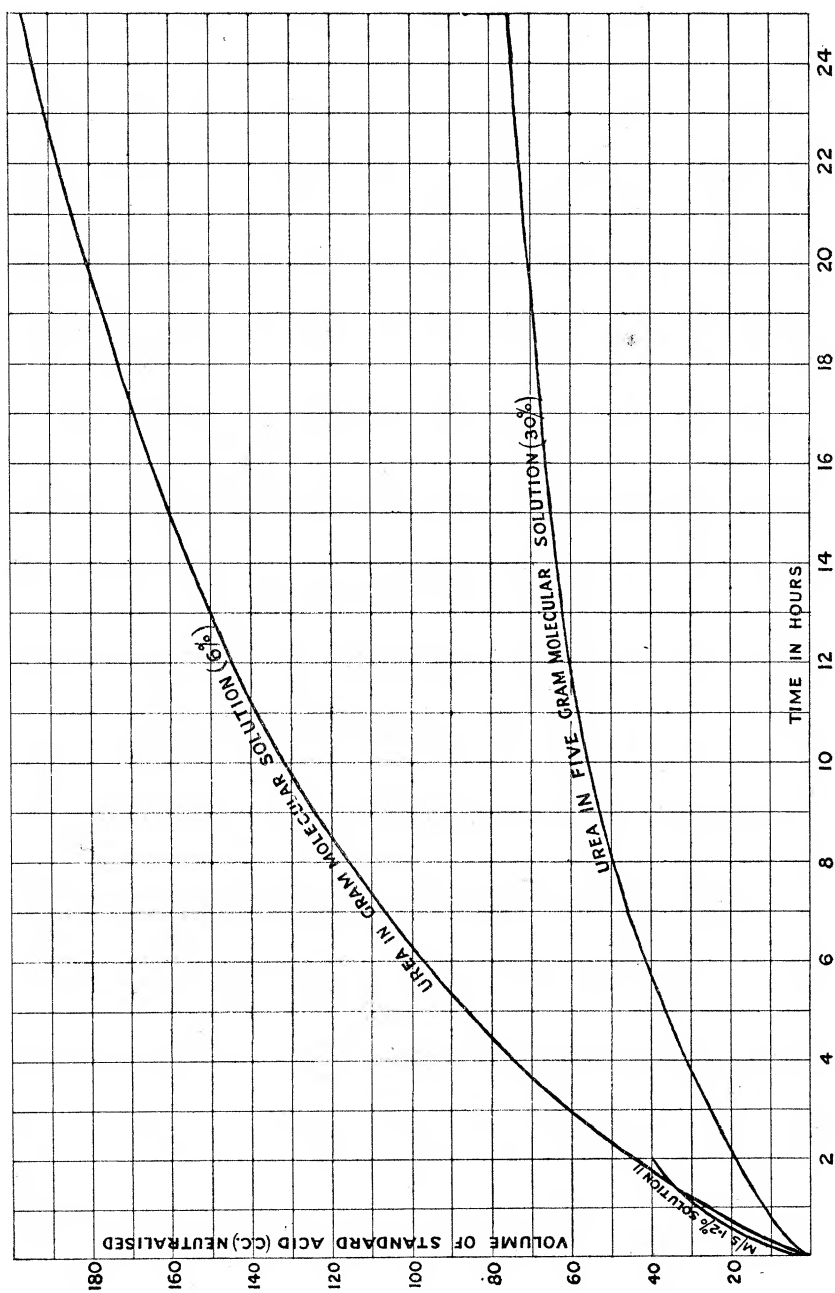
Time.	Percentage of urea hydrolysed	
	By 2 c.c. of Soja extract per 100 c.c.	By 4.5 c.c. of Soja extract per 100 c.c.
15 mins.	2.6	—
30 "	3.8	12.6
45 "	4.9	16.3
1 hour	6.1	21.3
1.5 "	7.9	28.4
2.0 "	8.6	35.0
2.5 "	9.7	40.9
3.0 "	11.9	46.4
4.0 "	13.0	56.8
4.5 "	13.8	61.3
5.0 "	14.3	65.4
6.0 "	16.2	73.7
6.5 "	17.1	77.2
24.0 "	37.1	100.0
144.0 "	52.9	

Table III.—Hydrolysis of Urea in different Concentrations by the same amount of Urease.*

Time.	Volume of standard acid solution neutralised by 10 c.c. samples of solution.		
	M/5 urea solution.	M urea solution.	5M urea solution.
5 mins.	c.c. 4.95	c.c. 4.2	c.c. 2.6
10 "	7.8	—	4.0
15 "	10.35	9.4	5.2
30 "	16.9	15.55	7.6
45 "	22.4	20.85	—
60 "	27.1	25.75	11.9
75 "	31.25	—	—
90 "	35.25	—	15.65
2 hours	39.14	—	19.1
3 "	—	61.8	25.4
5 "	—	85.35	36.4
7 "	—	108.4	46.2
25 "	—	196.85	—
49 "	—	—	93.8
Volume of acid neutralised by 10 c.c. on complete hydrolysis	39.5	197.5	987.5

* In these experiments, instead of boiling the solution after adding acid, the carbon dioxide was expelled by means of a current of air, in order to avoid the error arising from the hydrolysis of urea in concentrated solutions by the excess of acid.

was several times that effected during subsequent similar periods. Experiments with larger proportions of enzyme gave results which showed less disproportion (Table II). In the case of more concentrated solutions, on



increasing the concentration of the urea, the amount of action taking place was diminished rather than increased, little advantage being obtained by using solutions stronger than M/5. This remarkable result is brought out very clearly in Table III and in the graph on p. 117.

It will be noted that up to the time of complete hydrolysis of the urea present in the M/5 solution practically the same amounts of change took place in this solution and in that which was five times as strong; moreover, that in the still stronger 5M solution less than half as much urea underwent conversion as was hydrolysed in that of one-fifth the concentration.

Influence of the Products of Change.—As it appeared probable from these observations that the products of change were exercising a marked influence, experiments were made to test the influence of ammonia. The effect produced by this substance was found to be very considerable: not only was the rate of hydrolysis limited but the graphs representing the course of the change appeared flattened out from the outset, not curved as they are when no ammonia is added.

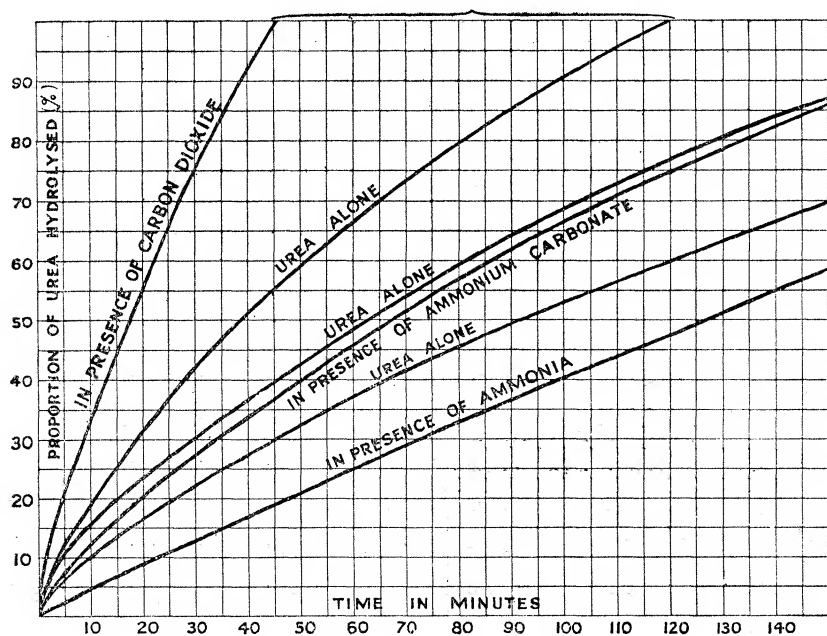
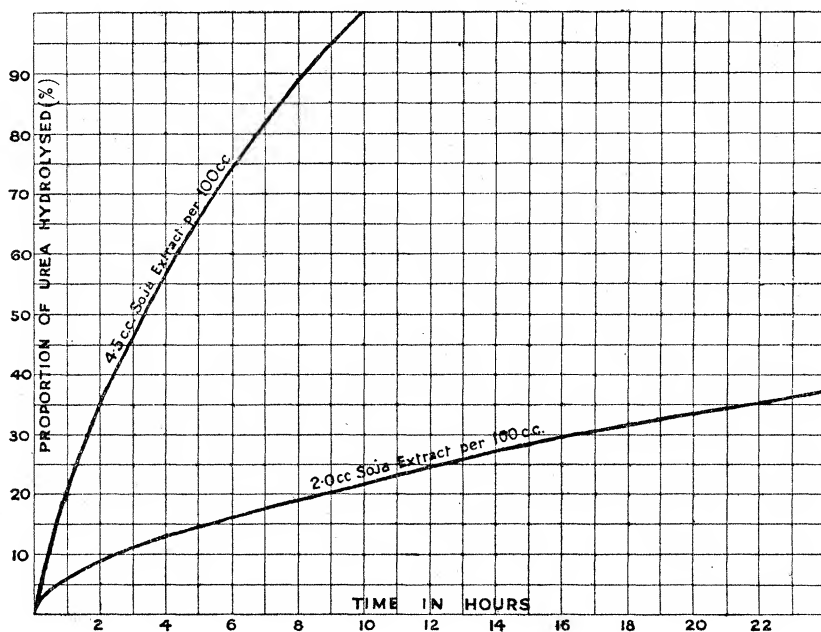
In an experiment in which 10 per cent. of the ammonia producible by the hydrolysis of the urea used was added at the outset, a small proportion of enzyme being used, the change followed an almost linear course during the first two hours, until over 50 per cent. was hydrolysed (Table IV, and the lowest pair of curves in the lower graph on p. 119).

Table IV.—Hydrolysis of Urea in the presence of Ammonia and Ammonium Carbonate.

Time.	Percentage of urea hydrolysed in solution containing			
	Urea alone.	Urea + ammonia.	Urea alone.	Urea + ammonium carbonate.
5 mins.	6·3	3·0	11·6	7·4
10 "	9·9	4·4	15·1	12·3
15 "	13·9	6·4	18·3	16·7
30 "	21·8	12·5	29·4	27·2
45 "	27·4	16·3	39·6	37·0
60 "	36·8	25·7	47·4	46·9
75 "	43·4	31·4	56·2	54·5
90 "	49·2	36·7	63·2	62·1
105 "	—	—	71·3	69·3
120 "	58·6	47·5	76·0	75·5
150 "	70·1	58·3	87·5	87·6
21 hours	97·3	97·6	95·5	95·5

When ammonic carbonate (equivalent to one-tenth of the urea used) was substituted for ammonia, though at first a decided retardation was obvious this was not so decided as in the case of ammonia and gradually (as

ammonia was formed) the two solutions approximated in behaviour—so that the change as a whole did not proceed at a linear rate. These results are indicated by the middle pair of curves in the lower graph on this page.



Again, when at the end of an experiment (when the action was complete and therefore the products of change were present in a proportion equivalent to the urea originally present) a fresh quantity of urea, equal to that originally taken, was added, the rate at which hydrolysis took place initially was about one-third of that first observed and the time required to effect complete hydrolysis was more than doubled.

In another experiment, in which a third portion of urea was introduced, hydrolysis took place at a very slow rate, 1·2 per cent. being changed after 30 minutes in this third stage instead of 10 per cent. in the second stage and 25 instead of 97 per cent. at the end of six hours.

Inasmuch as ammonia had so much more influence in retarding change than the carbonate, it appeared probable that the effect was attributable to its acting as an alkali. But it was conceivable that by favouring the activity of proteoclastic enzymes associated with the urease it might promote the destruction of the enzyme rather than retard its action.

Stability of the Enzyme.—To test the stability of the enzyme under various conditions, 25 c.c. of an extract prepared in the manner previously described was diluted to 250 c.c. with water; a second 25 c.c. was diluted to 250 c.c., sufficient ammonia being added to make the solution N/500 NH_3 in concentration; a third 25 c.c. was similarly diluted with water and acidulated to be of the strength N/500 HCl; a portion of the extract was set aside undiluted. The activity of each of these solutions towards urea was tested as soon as they were made and after various intervals and in the case of the acid and alkaline liquids, two experiments were made with each solution—

Table V.

Enzyme solution.	Percentage of urea hydrolysed in mixtures of 20 c.c. enzyme solution and 20 c.c. 2M/5 urea solution in 1 hour at 25°.							
	Initial.	1 day.	3 days.	4 days.	7 days.	11 days.	17 days.	60 days.
Aqueous solution of 10 per cent. of Soja extract [kept]	25·3	25·3	24·3	—	23·8	23·3	21·5	9·6
Ditto [made at time of experiment]	25·3	25·7	24·9	—	23·7	23·1	21·5	10·4
N/500 NH_3 solution of 10 per cent. of Soja extract [kept]	25·9	25·2	24·1	—	23·3	22·7	—	—
Ditto [neutralised at time of experiment]	25·9	25·3	24·6	—	22·7	23·7	22·8	11·0
N/500 HCl solution of 10 per cent. of Soja extract [kept]	25·9	25·0	21·4	19·6	17·3	15·5	—	—
Ditto [neutralised at time of experiment]	25·5	24·1	21·0	18·9	17·1	14·5	11·9	3·1

one with the unneutralised liquid, the other after neutralising it by adding the equivalent amount of alkali or acid. The results are given in Table V. It will be noticed that the acid but not the ammonia had a distinct influence in promoting decay of the enzyme.

The effect was found to be more marked in more strongly acid or alkaline solutions. The results given in Table VI show, however, that though the presence of ammonia influences the activity of the enzyme, decay takes place only gradually.

Table VI.

Enzyme solution.	Percentage of urea hydrolysed in mixtures of 20 c.c. enzyme solution and 20 c.c. 2M/5 urea solution in 1 hour at 25°.			
	Initial.	1 day.	2 days.	4 days.
Aqueous solution of 10 per cent. of Soja extract [kept]	26·4	24·9	25·3	21·4
N/25 HCl solution of 10 per cent. of Soja extract	2·6	—	—	—
N/25 NH ₃ solution of 10 per cent. of Soja extract [kept]	19·2	15·2	13·1	8·9
Ditto neutralised at time of experiment	—	—	—	8·8
Ditto saturated with CO ₂ at time of experiment.....	—	—	—	18·3

The experiments appear to justify the conclusion that the effects produced initially by ammonia are at least mainly due to the influence it exerts on the change itself and that there is no reason to suppose that the enzyme is destroyed by it, to any serious extent, either in the first instance or during the course of an experiment lasting a short time.

Influence of Carbon Dioxide.—Inasmuch as carbonate of ammonia has a very slight inhibiting effect in comparison with that of ammonia, it was to be supposed that carbonic acid would serve to neutralise, almost if not entirely, the effect ammonia produces. Having formed the opinion that the ammonia acted as an alkali and was in some way opposed to the urea, we therefore made the experiment in the full expectation that it would exercise a positive influence; we were not prepared, however, to find that carbonic acid has so marked an influence in promoting and increasing the activity of the enzyme as our experiments show that it has. The results of a pair of experiments are represented in the uppermost pair of curves in the lower graph on p. 119 and in Table VII.

In making these experiments, the behaviour of an M/5 solution of urea containing 25 c.c. of Soja extract was contrasted with that of a solution of the same strength prepared by saturating the liquid with carbon dioxide prior to adding the enzyme and then maintaining the passage of a current

Table VII.—Hydrolysis of Urea in the presence of Carbon Dioxide.

Time.	1st experiment. Percentage of urea hydrolysed in		2nd experiment. Percentage of urea hydrolysed in	
	Aqueous solution.	Carbonic acid solution.	Aqueous solution.	Carbonic acid solution.
mins.				
5	12·3	18·2	11·7	19·9
10	19·5	32·3	19·0	32·5
15	26·6	—	25·5	44·0
20	—	54·3	—	54·0
30	—	73·4	41·8	74·0
40	48·9	—	—	91·9
45	—	95·5	54·0	—
60	65·3	95·7	65·5	99·0
75	76·0	96·5	75·9	—
90	84·5	98·0	85·4	99·6
105	—	98·5	—	—
120	98·5	—	98·5	—

of the gas through the solution while it was heated in the incubator in proximity to the unheated solution.*

A similar effect is produced by the “amino-acid” glycine—thus the activity of a solution of the enzyme which produced 24 per cent. of change was raised to 35·3 per cent. by adding sufficient glycine to make it a 2N/5 solution of this substance.

Influence of Salts and Non-electrolytes.—As ammonia salts hasten whilst salts of the alkali metals retard the formation of urea from cyanate of ammonia, it was important to contrast the effect produced by ammonium

* *Note added February 23.*—The following series of results obtained recently are of interest as confirming those quoted in the communication, especially as the experiments were carried out simultaneously and with the same sample of enzyme. The very rapid rate of change in the presence of carbonic acid towards the close of the period of hydrolysis is noteworthy. A very striking diagram is obtained by plotting graphs from the figures given.

Percentage of Urea Hydrolysed in M/5 Solutions when alone or in presence of 1/10th equivalent of ammonic carbonate or 1/10th equivalent of ammonia, and in a solution saturated with carbon dioxide.

Minutes	5	10	15	20	25	30	40	45	50	60	75	90	120	150
Urea+ammonia	4·0	9·0	13·3	—	—	25·4	—	36·7	—	47·6	—	68·0	83·9	97·8
Urea+ammonic carbonate	8·6	16·4	22·2	—	—	37·6	—	48·8	—	59·4	—	79·8	96·8	—
Urea alone	11·8	19·2	25·5	—	—	41·5	—	54·4	—	65·1	74·4	83·2	97·0	—
Urea+carbon dioxide	20·9	30·0	42·3	54·2	65·0	75·9	98·5	—	99·3	—	—	—	—	—

and other chlorides on the hydrolysis of urea by urease. Potassium and sodium chloride act alike, both retarding the rate of change to a moderate extent. Ammonium chloride has a very slight accelerating effect, about equal to that exercised by glucose; methylurea, on the other hand, has as great an effect in retarding change as have potassium and sodium chloride. The various substances were present in the same molecular proportion as the urea at the outset. The results are represented in the set of graphs on p. 125 and in Table VIII.

Nature of the Process of Hydrolysis.—In view of the results we have obtained, there is reason to suppose that urease, like all other enzymes hitherto examined, is a selective enzyme—that is to say, an enzyme which fits the hydrolyte of which it is the specific hydrolyst. This hydrolyte may well be the hydrated form of urea, $C(OH)_2(NH_2)_2$, rather than urea itself.

The question to be considered is the nature of the change by which the urea is broken down. It seems to us that there is no reason to suppose that it is resolved otherwise than into carbonic acid and ammonia by a direct process of hydrolysis; the only intermediate product the formation of which should be considered is carbamic acid.

It has been argued by Fawsitt (*loc. cit.*) that the formation of carbonate from urea involves the intermediate conversion of the urea into cyanate but it may be contended that his argument is inconclusive and indeed irrational. In the light of modern knowledge, neither the conversion of urea into ammonic cyanate nor the reverse change can be looked upon as a simple process of internal molecular rearrangement; in both cases doubtless, a series of changes take place.

The formation of urea from the cyanate is probably a process involving both hydration and dehydration and may be represented, with some degree of probability, in the manner pictured in the equations on p. 112.

The formation of carbonic acid and ammonia from urea, on the other hand, is clearly a process of hydration and hydrolysis. It is conceivable and in our opinion probable that both changes—that into cyanate and that into carbonate—have their origin in the same substance, the hydrated compound which may be supposed to be present in a solution of urea $C(OH)_2(NH_2)_2$. Whilst this compound can give rise to cyanic acid, if deprived of ammonia and hydrone (OH_2); if hydrolysed, it can give rise to orthocarbonic acid and ammonia—

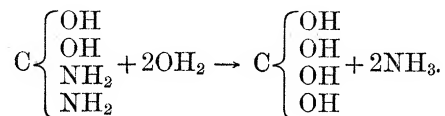
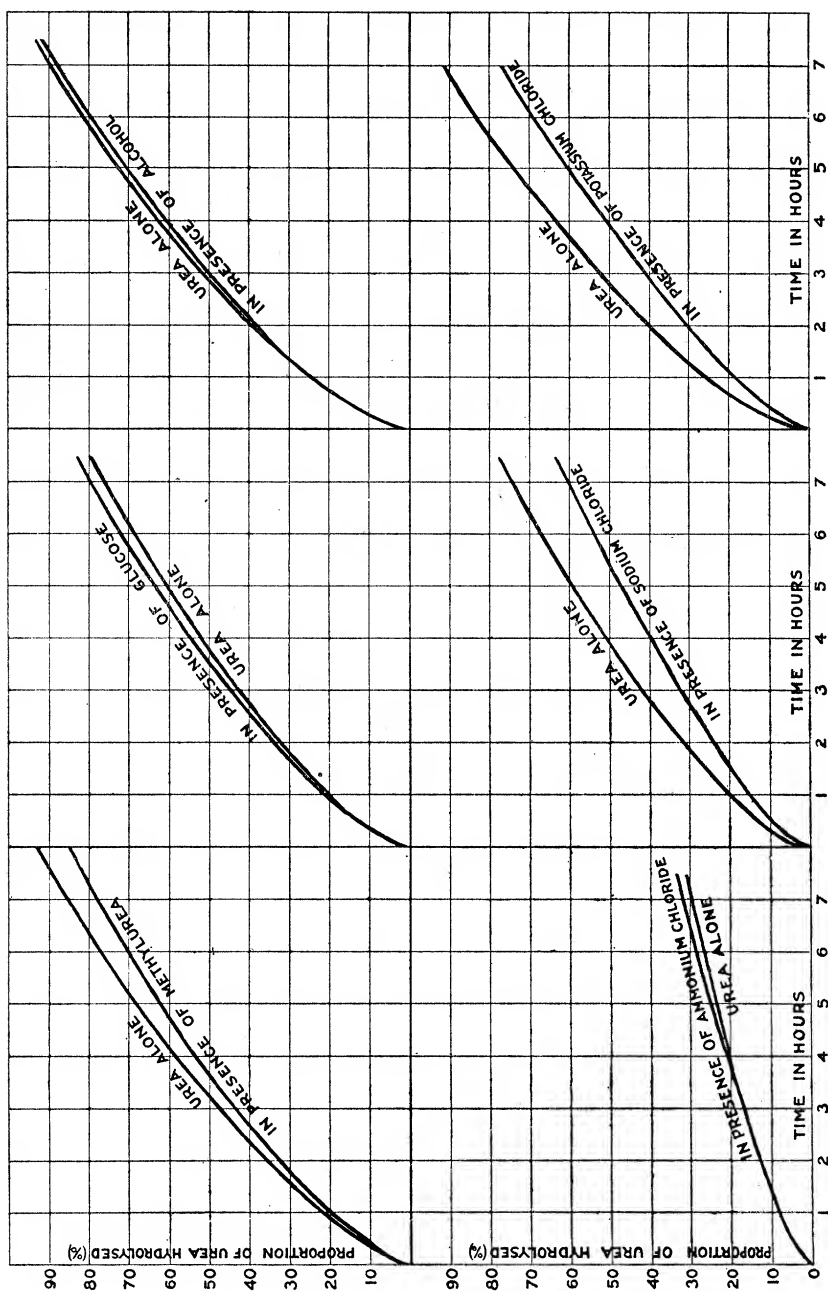


Table VIII.—Hydrolysis of Urea in the presence of Salts and Non-Electrolytes.

Time.	Percentage of urea hydrolysed									
	Alone.	In presence of methylurea.	Alone.	In presence of glucose.	Alone.	In presence of alcohol.	Alone.	In presence of NH_4Cl .	Alone.	In presence of NaCl .
hours.										
0.5	12.4	12.2	12.4	12.5	14.6	14.5	5.1	4.9	12.5	9.8
1.0	20.7	20.2	20.2	20.3	23.5	23.6	7.7	7.9	19.3	14.5
1.5	28.5	26.9	26.6	27.1	31.6	31.7	10.3	10.1	25.8	20.2
2.0	35.1	32.6	32.5	33.0	38.6	38.4	12.7	12.7	31.3	24.3
2.5	41.4	38.4	38.0	38.7	45.3	44.2	14.7	15.1	36.7	28.5
3.0	—	—	43.0	44.2	51.1	50.6	—	—	41.8	32.8
4.0	58.3	53.8	—	—	—	—	20.5	20.5	—	—
4.5	63.7*	58.2	56.9	58.5	67.0	66.1	22.2	22.4	54.5	43.4
5.0	67.8	62.3	61.0	63.1	72.1	71.3	23.9	24.4	58.8	47.2
5.5	72.4	66.4	65.2	67.0	76.0	75.5	25.2	26.5	62.8	50.6
6.0	77.0	70.8	69.3	73.5	80.6	80.1	26.0	27.4	66.5	53.6
6.5	80.9	74.5	72.9	75.2	84.8	84.7	28.1	29.7	70.1	57.0
7.0	84.9	78.5	76.6	78.8	89.2	88.1	—	—	73.9	60.1
7.5	—	—	80.1	81.9	93.0	92.2	31.2	33.0	90.7	63.4
8.0	92.3	84.7	—	—	—	—	70.4	73.1	—	—
24.0	99.8	100.7	—	—	99.0	99.2	—	—	99.2	99.4
24.5	—	—	99.9	99.9	—	—	—	—	99.6	—

* Sample taken after 4 hrs. 35 mins.



Hydrolysis of Urea in presence of Salts and Non-Electrolytes.

The function of urease would seem to be to determine the change in this latter direction: in other words, to condition the direct hydrolysis of urea and thereby prevent its reversion into cyanate.

It is necessary to account, on this assumption, for the effects produced by salts and non-electrolytes. The marked inhibiting effect of ammonia and the slight effect produced by ammonium chloride in hastening hydrolysis are hardly to be accounted for on the assumption that these compounds directly affect the stability of the urea: if we are not mistaken, according to current views, it is rather to be supposed that the ammonium salt would exercise the greater effect in repressing hydrolysis and the alkali ammonia should tend to promote hydrolysis.

We are inclined to think that an explanation is to be found by regarding the enzyme as a feebly acidic substance and that, in order that it may condition the change, it must unite with the feebly basic substance urea—the effect of a more basic substance such as ammonia would be to interfere with such union and consequently to retard change. Carbonic acid, by fixing the ammonia, would tend to promote the action of the enzyme from this point of view by leaving it free to act as hydrolyst. To account for the extraordinary retardation effected by urea itself, it must be supposed that the alterations in the osmotic conditions which are the consequence of an increase in the concentration of the solution in some way affect the rate at which change can take place; it may be that the proportion of urea effectively hydrated diminishes as the concentration is increased—that is to say that the compound $C(OH)_2(NH_2)_2$ is formed in diminishing proportion as the concentration is increased. The influence of non-electrolytes is at least in part to be accounted for on this assumption.

Urea is to be regarded as a compound which has very little tendency under ordinary conditions to become carbonate. Apparently it is protected from decomposition by its tendency to become cyanate; on the other hand, the cyanic acid which is formed from it when the change occurs is protected by the presence of an equivalent proportion of ammonia and is protected also by its tendency to revert to urea. In other words, both urea and cyanate are protected from further change by the tendency each has to become the other. The production of a relatively large amount of carbonate when ammonic cyanate is taken instead of urea is perhaps due to the fact that the solution then contains a relatively much larger amount of free cyanic acid than is present in a solution of urea, cyanic acid (in absence of ammonia) being an unstable substance which is readily hydrolysed to carbonic acid. That is to say, carbonic acid may be formed in two ways, from cyanic acid and from urea: under normal conditions, in a solution of urea neither the cyanic acid nor the urea-hydrate which are contained in the solution undergoes decomposition into carbonic acid to an extent which is appreciable, being

present only in minute amounts. Urease, by determining the resolution of the urea in an abnormal direction therefore serves a most important purpose.

In studying the phenomena, the mistake has been made hitherto of treating the changes attending the formation and destruction of urea from a point of view which has involved under-rating their complexity.

We venture to think that the peculiarities to which we call attention are not only noteworthy but should serve as a warning against the danger attending the attempt to formulate conclusions as to the nature of chemical changes without giving due weight to chemical considerations—a practice too frequently followed in these days.

It is proposed to extend the inquiry.

[The cost of this investigation has been in part met by a grant for which I am indebted to the Government Grant Fund of the Royal Society.—H. E. A.]

*Certain Results of Drying Non-sporing Bacteria in a Charcoal
Liquid Air Vacuum.*

By S. G. SHATTOCK, F.R.C.S., and L. S. DUDGEON, F.R.C.P.

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The following experiments were undertaken with the object of ascertaining whether non-sporing bacteria, dried *in vacuo* and kept *in vacuo*, would survive those dried in and kept in the air, or, on the contrary, whether they would die more rapidly. The action of sunlight and of heat was tested, moreover, upon bacteria dried *in vacuo* and kept *in vacuo*, with a view of discovering how far such agencies might be lethal upon dried bacteria, if the latter were supposed present in a free state in interplanetary space.

In carrying out the work, we have had the invaluable advantage of Sir James Dewar's help, for of the several methods of drying *in vacuo*, by far the most efficient is that devised by him. This method is so well known to physicists that it will be enough to state here, that after the air of the vessel is exhausted by means of an air pump, the glass connection with the pump is sealed off in the blowpipe flame, and the exhausted chamber is deprived of its remaining gases through a second outlet communicating with a bulb containing cocoanut charcoal (previously freed from gases),